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FUNCTIONAL PROPERTIES OF LEGUME PROTEINS MODIFIED BY ESTRIFICATION

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ABSTRACT: At three legume proteins i.e. soybean protein isolate (SPI), broad bean protein isolate (BPI) and chick pea protein isolate (CPI) were esterified to different extents with methanol, the esterified proteins were analysed for solubility, emulsifying and foaming properties at a pH range of 2-10. These functional properties were changed in the esterified proteins compared to the native ones. The magnitude of change depended on the extent of esterification and the nature of the modified protein. Emulsifying activity and stability of esterified legume proteins at the acidic pH-range of 2-6 were generally higher compared to the corresponding native proteins. The foam activity and stability at the pH-range 2-6 were generally higher compared to the corresponding native proteins. An improvement was associated with the degree of solubility, the enhanced degree of esterification and the nature of the used protein.

Key words: Soybean protein isolate, broad bean protein isolate, chick pea protein isolate, functional properties and esterification.

INTRODUCTION

The plant seeds are not only an organ of propagation and dispersal but also the major plant tissue harvested by humankind. The amount of protein present in seeds varies from ~10% (in cereals) to ~40% (in certain legumes and oilseeds) of the dry weight, forming a major source of dietary protein. Legumes have historically been utilized mainly as whole seeds. However, in recent years, interest has grown in the utilization of legumes in other forms (e.g. like flour, concentrate and isolate) rather than the whole seeds (Doxastakis, 2000 and Saio, 1993). Soy proteins isolates (SPI) consist of about 90% protein, their major components are glycinin, or 11S

and beta-conglycinin, or 7S, which represent 34% and 27% respectively, of the proteins occurring in the isolate (Iwabuchi and Yamauchi, 1987a, and b). The similarity between protein components of soybean and those of other legumes (Barker, *et al.,* 1976 and Derbyshire, *et al.,* 1976) suggests that they may have similar functions and applications. The storage proteins, 7S (β-conglycinin) and 11S (glycinin), are the principal components of soybean proteins (Kinsella, 1979), whereas 7S (Vicilin) and 11S (Glycinin), are the principal components of other legume proteins (Derbyshire *et al.*, 1976).

Chemical modification of native proteins is one of the first methods employed to investigate structure-function relationships. Esterification is an important and easy tool of modification of proteins.Esterification blocks free carboxyl groups raising thus the net positive charge and rendering more basic the modified protein (Halpin and Richardson 1985; and Sitohy, *et al.,* (2001). Vital functional properties required in protein ingredients include solubility, emulsifying properties, foaming properties. Since good solubility and emulsification properties at acidic pHs are required for some food application such as acid soft drinks and acid foods, esterification of proteins improving their functional properties in this pH range may be required. Estereification of proteins was proved to block the protein negative charges increasing thus the net positive charge and raising the protein isoelectric points. This should render the modified proteins more soluble and likely much more tension-active in the acidic range of pH.The isoionic point of β-lacto globulin was raised from 5.2 to 6.2, 8.7 and 9.8 after its esterification with butanol, ethanol and methanol, respectively (Halpin and Richardson, 1985; and Mattarella and Richardson, 1983). The aim of this work was to determine if the functional properties of SPI, BPI and CPI are improved after this modification so that they can favorably be incorporated as food hydrocolloids.

MATERIALS AND METHODS

Materials

Plant Materials

Soybean (*Glycine max* L.), chick pea (*Cicer arietinum* L.) and

broad bean (*Vicia faba* L.) seeds were purchased from local market, Zagazig City, Sharkia, Egypt.

Chemicals

Methyl alcohol and hydrochloric acid were obtained from Merk, Germany. All chemicals used in experiments were of analytical grade.

Methods

Sample Preparations

Soybean, broad bean, and chick pea seeds were manualy cleaned and ground for 3min using a Moulinex mixer (Type 716, France) at a maximum speed and the meal were ground to pass through a 1mm² sieve. The powder was then defatted using chloroform: methanol (3:1v/v) for 8h. Solvent was evaporated by rotary-evaporator and dried-defatted meal was stored at 4°C until analysis.

Extraction of Protein Isolates

Dispersions of 5% (w/v) defatted soybean, broad bean, or chick pea flour in water were adjusted to pH 9 with 0.1 N NaOH at room temperature, shaken for 1h and centrifuged for 15min at 2000g. In order to obtain increased yields, the extraction and centrifugation procedures were repeated on the residue. The extracts were combined and the pH adjusted to 4.5 with 1N HCl to precipitate the protein. The proteins were recovered by centrifugation at 2000g for 15min followed by removal of the supernatant by decantation. Curde protein was washed with distilled water and the curde was dispersed in distilled water at pH 7.5, dialyzed overnight and lyophilized. (Johnson and Brekke 1983).

Determination of Protein Content

Total protein content of SPI, BPI and CPI were calculated by multiplying the total nitrogen by 6.25. The total nitrogen was determined by using micro Kjeldahel method according to A.O.A.C. (1996).

Protein Esterification

The procedure of Sitohy *et al.* (2000) was used. SPI, BPI, or CPI were esterified by dispersing them in concentrated (>99.5%) methyl alcohol (5% w/v). Amount of hydrochloric acid equivalent to 50M ratio (MR, mol acid/mol carboxyl group), applied to induce the protonation of carboxylates, were added drop- wise at the start of the reaction. All the reaction

mixtures were kept at 4°C under continuous stirring.At the end of the reaction (10 h), the samples were centrifuged at 10000g for 10min. The resulting supernatant was discarded and the residue was dispersed in a volume of alcohol equal to that of the discarded supernatant and well mixed before re-centrifuging in the same conditions . The washing step was repeated three times. The final precipitate was dissolved in an appropriate amount of distilled water at pH 7.5, dialyzed overnight and lyophilized. The lyophilized samples were kept at –20°C until analysis.

Esterification Extent

The extent of esterification of proteins was quantified by the colour reaction with hydroxylamine hydrochloride as developed by Halpin and Richardson (1985) and modified by Bertrand-Harb *et al.* (1991).

Functional Properties of Native and Modified Proteins

pH-solubility profile

One hundred and twenty-five milligrams of the samples were dispersed in 25ml of distilled water and the solution pH was adjusted to 2-10 using either 0.5mol/L NaOH or 0.5 mol/L HCl. The slurries were mixed for 1h at 30°C using magnetic bar before centrifuging at 1200g for 20 min at 4°C. The supernatant was filtered to obtain a clear solution. Protein content in the supernatant was determined by Kjeldahl method (A.O.A.C, 1996). Triplicate determinations were carried out and the solubility profile was obtained by plotting averages of protein solubility (%) against pH:

$$\text{Solubility (\%)} = \frac{\text{Amount of protein in the supernatant}}{\text{Amount of protein in the sample}}$$

Emulsifying activity and stability

Emulsifying activity and stability were determined using the method of Neto *et al.* (2001). Five milliliters portions of protein solution (2%w/v) were homogenized with 5 ml corn oil. The emulsions were centrifuged at 1100g for 5min. the height of emulsified layer and that of the total contents in the tube was measured.

The emulsifying activity (EA) was calculated as:

$$\text{EA (\%)} = \frac{\text{Height of emulsified layer in the tube x 100}}{\text{Height of the total contents in the tube}}$$

Emulsion stability was determined by heating the emulsion at 80°C for 30min before centrifuging at 1100g for 5min as:

$$ES\ (\%) = \frac{\text{Height of emulsified layer after heating x 100}}{\text{Height of emulsified layer before heating}}$$

Influence of pH on emulsifying activity and stability was investigated by preparing protein solutions of various pHs ranging from 2 to 10.

Foaming properties

The foaming capacity and stability were studied according to the method of Coffman and Garcia (1977). Weighted amount of protein isolate was dispersed in 100ml-distilled water. The resulting solution was whipped vigorously for 2min in a Moulinex mixer (Type 716, France) at the maximum speed. Volumes were recorded before and after whipping. The percentage volume increase was calculated according to the following equation:

$$\% \text{ Volume} = (V_2\text{-}V_1)/(V_1) \times 100,$$

Where V_2 is the volume of protein solution after whipping and V_1 the volume of protein solution before whipping.

Foam stability was detrmined as the volume of foam that remained after 8hr at room temperature expressed as a percentage of the initial foam volume.

Influence of pH on foam capacity and stability was investigated by preparing protein solutions of various pHs ranging from 2 to 10.

RESULTS AND DISCUSSION

Protein Content of Protein Isolates

Data for the total protein (calculated as g/100g dry matter) of SPI, BPI and CPI are presented in Table 1. The highest total protein content was found in CPI (91.88%) followed by SPI (90.71%) and BPI (90%).

Table 1. Total protein of protein isolates (% on basis of dry matter)

Protein isolate	%Protein
SPI	90.71
BPI	90
CPI	91.88

Esterification Extent

The color reaction using hydroxylamine hydrochloride was used with modification to quantify the extent of esterification of

proteins and the results are shown in Table 2. The highest level of esterification was recorded for methylated chick pea protein isolate (MCPI, 83.47%) followed by methylated soybean protein isolate (MSPI, 79.19%) and methylated broad bean protein isolate (MBPI, 74.9%).

Table 2. The extent of esterification of protein isolates (%)

Protein isolate	Esterification extent (%)
MSPI	79.19
MBPI	74.9
MCPI	83.47

Functional Properties of Native and Modified Proteins

pH-solubility profile

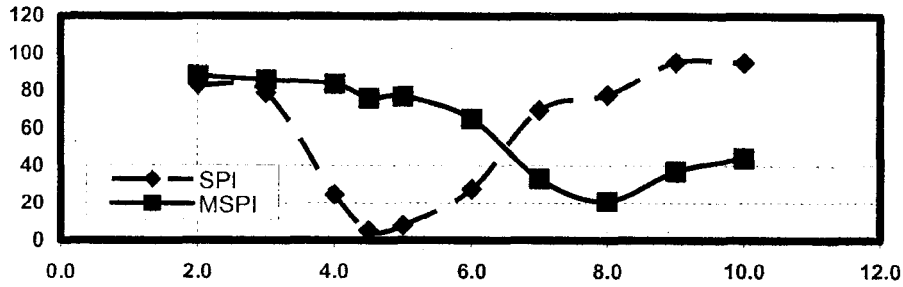
Solubility is one of the most important characteristics of proteins because it is not only important by itself, but also influences other functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gels, because soluble proteins provide a homogenous dispersibility of the molecules in colloidal systems and enhance the interfacial properties (Zayas, 1979).

The pH-solubility curve of native and esterified SPI is presented in Fig.1-a.The solubility profile of native SPI indicate that protein solubility reduced as the pH increased from 2 to 4.5.which correspond to its isoelectric point, after which subsequent increase in pH increased protein solubility progressively. The minimum solubility for native SPI (5.2%) was at pH 4.5. which corresponds to its isoelectric point (IEP). The highest protein solubility (95.2%) was observed at pH 10.

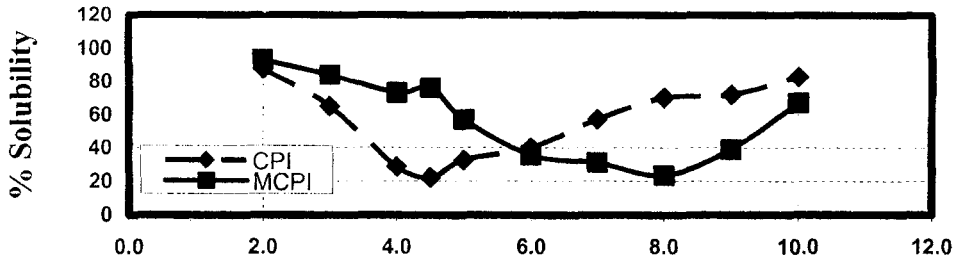
Esterification increased protein solubility in the acidic pH rang from 6 to 2. Increasing the pH higher than 7 reduced gradually the solubility giving a minimum value (21.2%) at pH 8. Esterification improved the solubility of the unmodified protein isolate at the isoelectric point. MSPI was more soluble in the acidic range of pH and less soluble in the alkaline range of pH as compared to unmodified protein.

The pH solubility curve of native and esterified CPI is presented in Fig. 1-b. The solubility profile of native CPI indicate that protein solubility reduced as the pH increased from 2 to 4.5, which correspond to its isoelectric point, after which

(a) Soybean protein



(b) Chickpea protein



(c) Broad bean protein

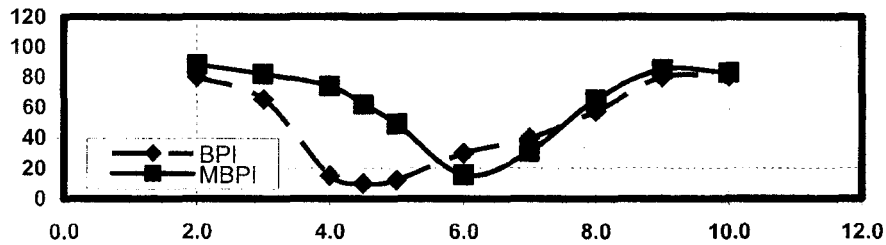


Fig.1. Protein solubility profile of native and esterified legume proteins at different pH values

subsequent increase in pH increased protein solubility progressively. The minimum Esterification increased protein solubility in the acidic pH rang from 2 to 5. Increasing the pH higher than 6 reduced gradually the solubility giving a minimum value (23.5%) at pH 8. Esterification improved the solubility of the unmodified protein isolate at the isoelectric point. MCPI was more soluble in the acidic range of pH and less soluble in the alkaline range of pH as compared to unmodified protein.

The pH solubility curve of native and esterified BPI is presented in Fig. 1-c. The solubility profile of native BPI indicate that protein solubility reduced as the pH increased from 2to4.5, which correspond to its isoelectric point, after which subsequent increase in pH increased protein solubility progressively. The minimum solubility for native BPI (9.9%) was at pH 4.5 which corresponds to its isoelectric point (IEP). The highest protein solubility (80.5%) was observed at pH10.

Esterification increased protein solubility in the acidic pH rang from 2 to 5. Increasing the pH higher than 6 reduced gradually the solubility giving a minimum value (15.7%) at pH 6. Esterification improved the solubility of the unmodified protein isolate at the isoelectric point. MBPI was more soluble in the acidic range of pH and less soluble in the alkaline range of pH as compared to unmodified protein.

Sitohy *et al.* (2001) recorded that solubility of β-lacto globulin esters depends on the degree of esterification as well as on the nature of the grafted ester groups. Samples of highly esterified β-Lacto globulin (99%) gave rise to more homogenous protein population with a minimum of solubility near pH10, while those with low degrees of esterification gave heterogonous population showing two solubility minima. Consequently, two opposite effects of esterification on the solubility of β-Lacto globulin in the acidic pH range could be perceived. The first effect improving solubility results from the isoelectric point shift towards the alkaline pH.The second effect lowering solubility results from replacing hydrophilic carboxylic groups by hydrophobic ester groups. The overall protein solubility results from the interplay

between these two effects. The first effect is more efficient in case of the most esterified derivatives and when the grafted ester group is of a less hydrophobic nature (e.g. propyl). This may explain the reduced solubility reported in case of some esterified proteins at acid pHs (Mattarella *et al.,* 1983). The conformation of the esterified protein might play an important role on the solubility of the protein. Hence; it is not only the nature of the grafted ester group that determines the solubility of the modified protein but also the nature of the protein itself and the pH degree.

Emulsifying properties

Effect of pH on emulsifying activity and emulsion stability is a reflection of the influence of pH on protein solubility.

Effect of pH on emulsifying activity of native and methylated SPI is presented in Fig. 2-a. The maximum emulsifying activity of native SPI (83.3%) was obtained at pH 10 of the protein solution. Emulsifying activity decreased with increase in pH until it reached minimum value (13.6%) at pH 5. Among the methylated derivatives, the minimum emulsifying activity was recorded at pH 8 (22.6%) which were generally lower compared with native protein isolate. The maximum emulsifying activity of MSPI (97.5%) was obtained at pH 6. Esterification increased emulsifying activity in the acidic pH range from 2 to 6. Increasing the pH higher than 6 reduced gradually the emulsifying activity giving a minimum value at pH 8. The behavior of emulsion stability was also pH dependent (Fig.3-a). At pH 5 native SPI had the minimum emulsion stability of 20%, followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of native SPI (86.8%) was obtained at pH10. Esterification increased emulsion stability in the acidic pH range from 2 to 6. Increasing the pH higher than 6 reduced gradually the emulsion stability giving a minimum value at pH 8 (35%). The maximum emulsion stability of MSPI (96.8%) was obtained at pH 5.

Effect of pH on emulsifying activity of native and MCPI is presented in Fig. 2-b. The maximum emulsifying activity of native CPI (72.5%) was obtained at pH 10 of the protein solution. Emulsifying activity decreased with increase in pH until it reached

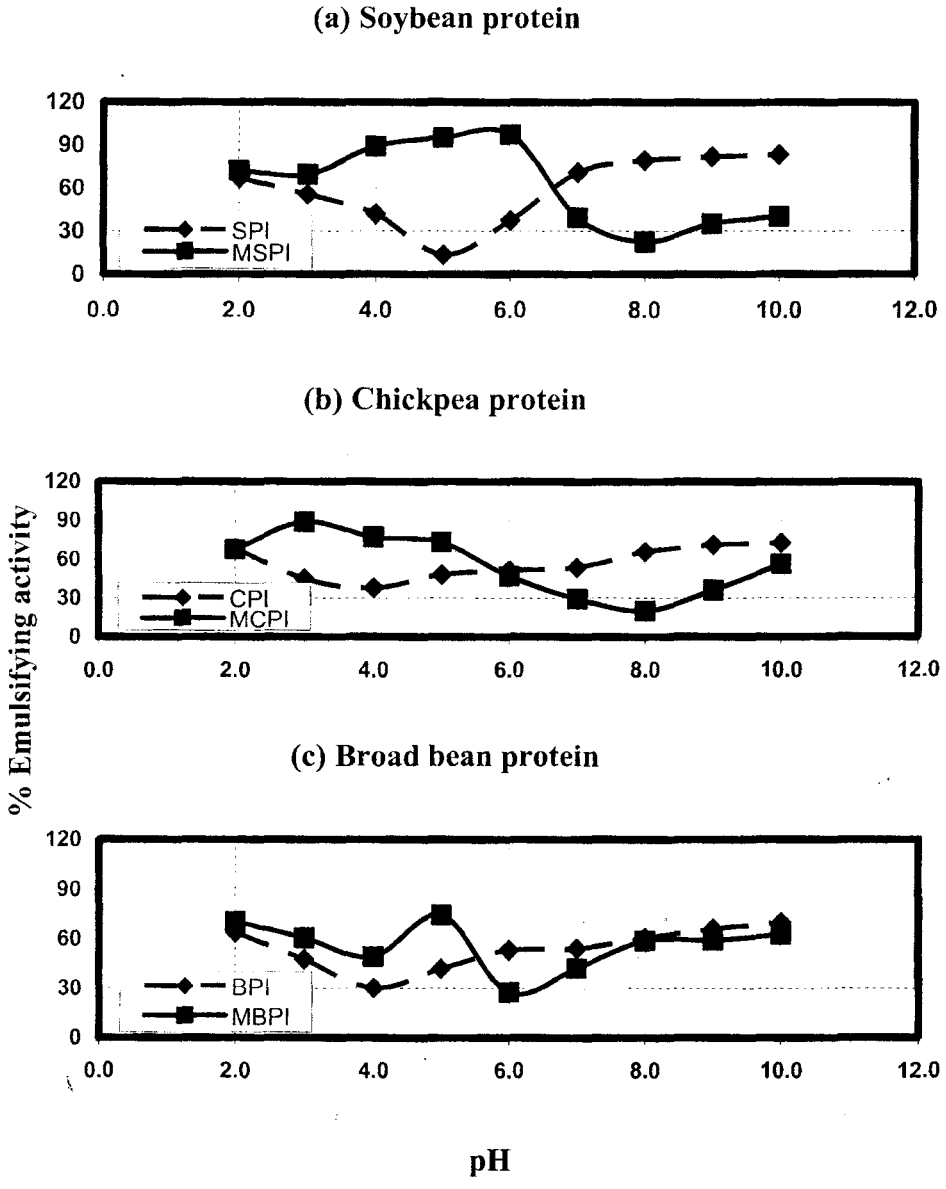


Fig.2. Emulsifying activity of native and esterified legume proteins at different pH values

minimum value (37.8%) at pH 4. Among the methylated derivatives, the minimum emulsifying activity was recorded at pH 8 (20.3%) which were generally lower compared with native protein isolate. The maximum emulsifying activity of MCPI (88.6%) was obtained at pH 3. Esterification increased emulsifying activity in the acidic pH range from 2 to 5. Increasing the pH higher than 5 reduced gradually the emulsifying activity giving a minimum value at pH 8 (20.3%). The behavior of emulsion stability was also pH dependent (Fig.3-b.)

At pH 4 native CPI had the minimum emulsion stability of 45.6%, followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of native CPI (80.6%) was obtained at pH10. Esterification increased emulsion stability in the acidic pH range from 2 to 6. Increasing the pH higher than 6 reduced gradually the emulsion stability giving a minimum value at pH 8 (37.7%). The maximum emulsion stability of MCPI (92.2%) was obtained at pH 4.

Effect of pH on emulsifying activity of native and methylated BPI is presented in Fig. 2-c. The maximum emulsifying activity of native BPI (70%) was obtained at pH 10 of the protein solution. Emulsifying activity decreased with increase in pH until it reached minimum value (30.3%) at pH 4. Among the methylated derivatives, the minimum emulsifying activity was recorded at pH 6 (27.7%) which were generally lower compared with native protein isolate. The maximum emulsifying activity of MBPI (74.7%) was obtained at pH 5. Esterification increased emulsifying activity in the acidic pH range from 2 to 5. Increasing the pH higher than 5 reduced gradually the emulsifying activity giving a minimum value at pH 6 (27.7%) Fig. 2-c.

At pH 4 native BPI had the minimum emulsion stability of 30.2%, followed by subsequent increase in emulsion stability as the pH increased. The maximum emulsion stability of native BPI (67.4%) was obtained at pH10. Esterification increased emulsion stability in the acidic pH range from 2 to 5. Increasing the pH higher than 5 reduced gradually the emulsion stability giving a minimum value at pH 6 (23.7%). The maximum emulsion stability of MBPI (73.6%) was obtained at pH 5.

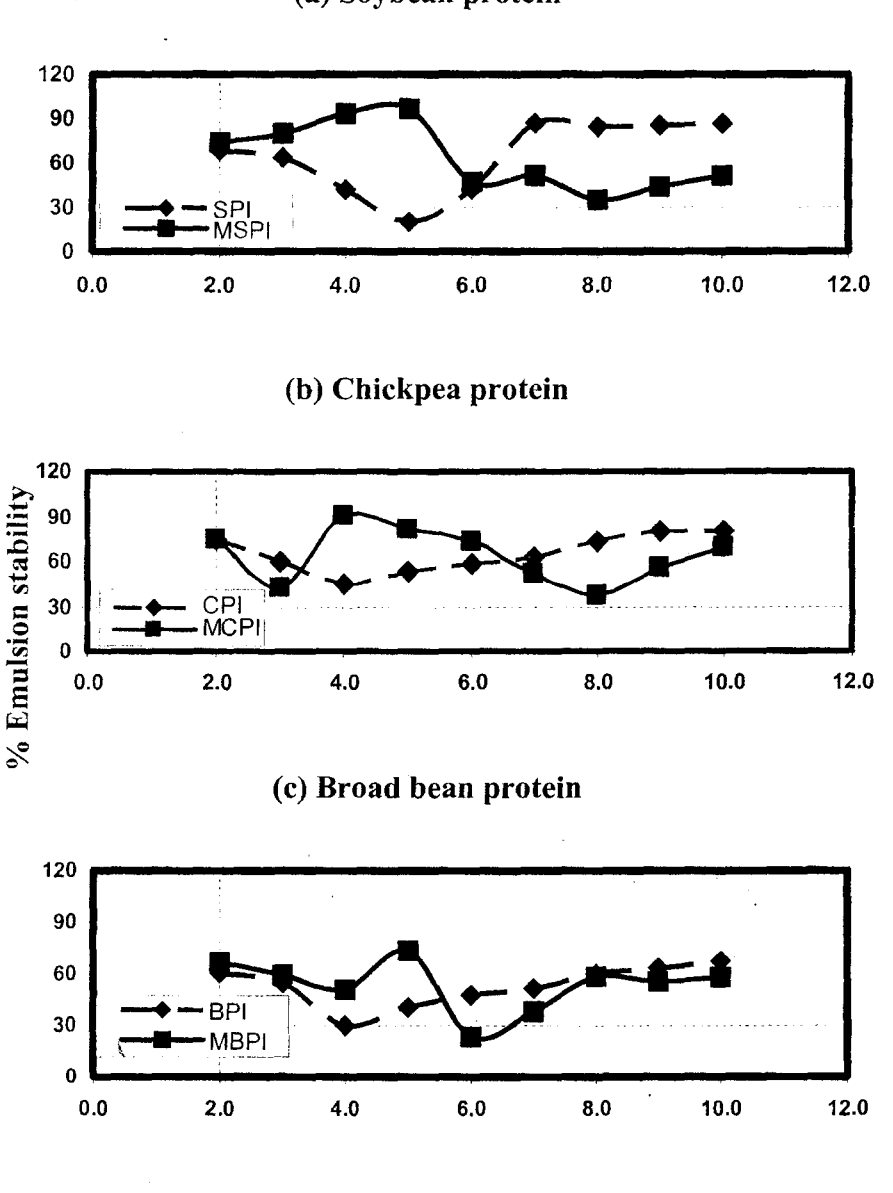


Fig.3. Emulsion stability of native and esterified legume proteins at different pH values

Dependence of emulsion activity on pH was expected as it is known that emulsifying activity of soluble proteins depend upon the hydrophilic-lipophilic balance (Sosulski and Fleming, 1977), which is affected by pH.At the oil-water interface, the protein orients lipophilic residues to the oil phase and hydrophilic residues to the aqueous phase, thus reducing surface tension at the interface. Esterification enhanced exposure of lipophilic and hydrophilic residues and this improved emulsifying activity.

Foaming properties

Effect of pH on foam capacity of native and methylated SPI is presented in Fig.4-a. Native SPI recorded 131.3% foam capacity at pH 10 and this reduced to 27.3% at pH 5, where minimum value was observed. For methylated protein, minimum foam capacity (28%) was observed at pH 8. Maximum foam capacity (124.6%) was observed at pH5. Marked improvement in foam capacity (Fig. 4-a) of native protein at pH 2, pH9 and pH10 is a result of enhanced solubility at these conditions. More so, the trend of increase in foam capacity agrees with the pattern of pH dependent solubility profile Fig.1-a.

Effect of pH on foam stability of native and methylated SPI is presented in Fig.5-a. Native SPI had a foam stability of 29.3% at pH7, and this increased to 102% at pH 4. Among the methylated protein, the maximum foam stability of the methylated protein was at pH4 (106%). Esterification generally increased the foam stability of SPI at pH range from 2 to 6. And reduced it at pH range from 7 to10. Increase in the foam stability at region of IEP is due to the formation of stable molecular layers in the air-water interface of the foams. Protein adsorption and viscoelasticity at an air-water interface is maximum near or at isoelectric pH because a protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air – water interface, a development that improves foam stability. This observation lends credence to similar results that have been reported earlier (Buckingham, 1970).

Effect of pH on foam capacity of native and methylated CPI is presented in Fig.4-b. Native CPI recorded 116.7% foam capacity at pH 10 and this reduced to 48% at

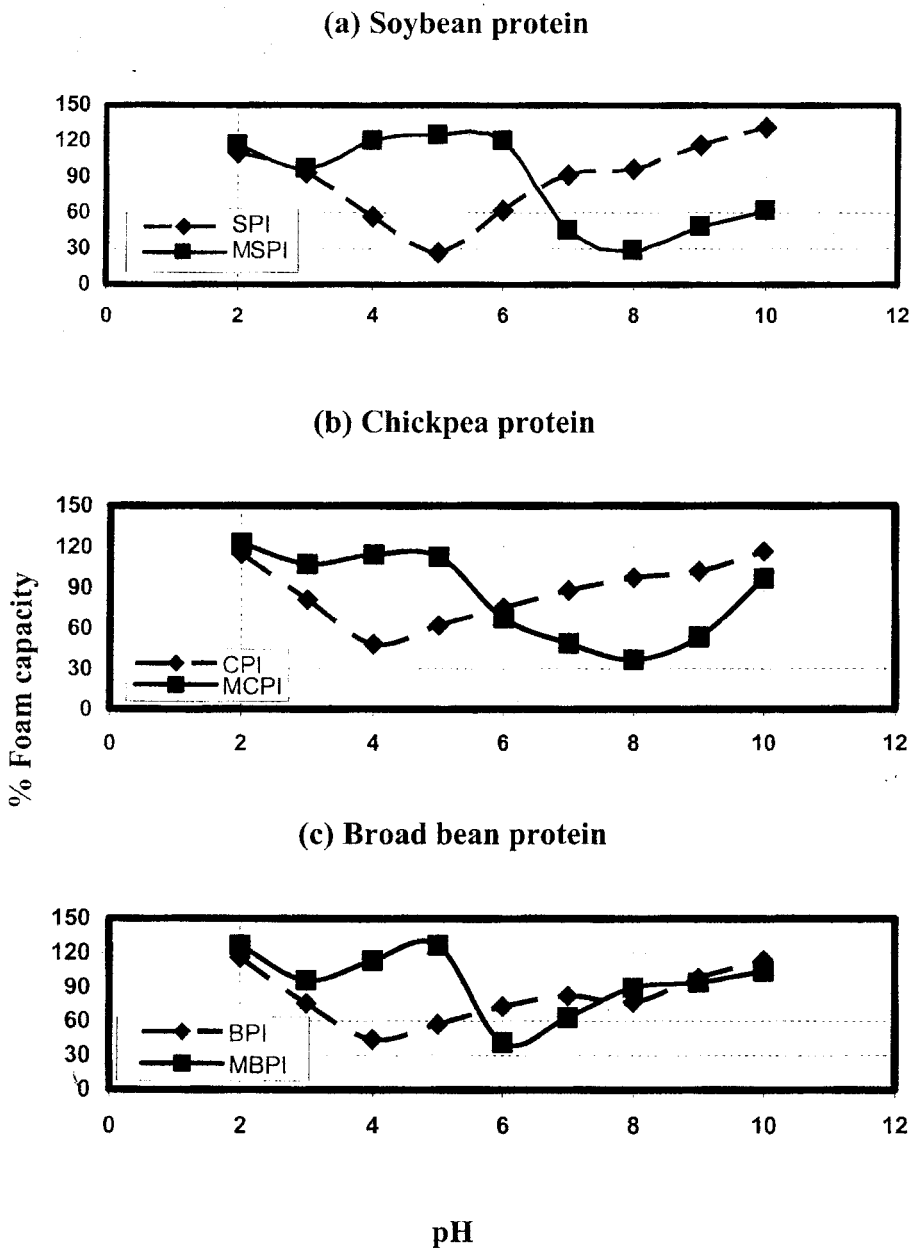


Fig.4. Foam capacity of native and esterified legume proteins at different pH values

pH 4, where minimum value was observed. For methylated protein, minimum foam capacity (36.7%) was observed at pH 8. Maximum foam capacity (122.7%) was observed at pH2. Marked improvement in foam capacity (Fig. 4-b) of modified protein at pH 3, pH4, pH 5 and pH10 is a result of enhanced solubility at these conditions. More so, the trend of increase in foam capacity agrees with the pattern of pH dependent solubility profile (Fig. 1-b).

Effect of pH on foam stability of native and methylated CPI is presented in Fig.5-b. Modified CPI had a foam stability of 23.3% at pH8.and this increased to 108.7% at pH 3. Among the methylated protein, the maximum foam stability of the methylated protein was at pH4 (96%). Esterification generally increased the foam stability of CPI at pH range from 2 to 5 and reduced it at pH range from 6 to10.

Effect of pH on foam capacity of native and methylated BPI is presented in Fig.4-c. Native BPI recorded 116% foam capacity at pH 2 and this reduced to 44% at pH 4, where minimum value was observed. For methylated protein, minimum foam capacity (41.3%) was observed at pH 6. Maximum foam capacity (126.6%) was observed at pH2 and pH5. Marked improvement in foam capacity (Fig.5-a) of native protein at pH 2, pH9, and pH10 is a result of enhanced solubility at these conditions. More so, the trend of increase in foam capacity agrees with the pattern of pH dependent solubility profile (Fig.1-c).

Effect of pH on foam stability of native and methylated BPI is presented in Fig.5-c. Modified BPI had a foam stability of 30% at pH6. And this increased to 94% at pH 4. Among the methylated protein, the maximum foam stability of the methylated protein was at pH4 (94%). Esterification generally increased the foam stability of BPI at pH range from 2 to 5 and reduced it at pH range from 6 to 10.

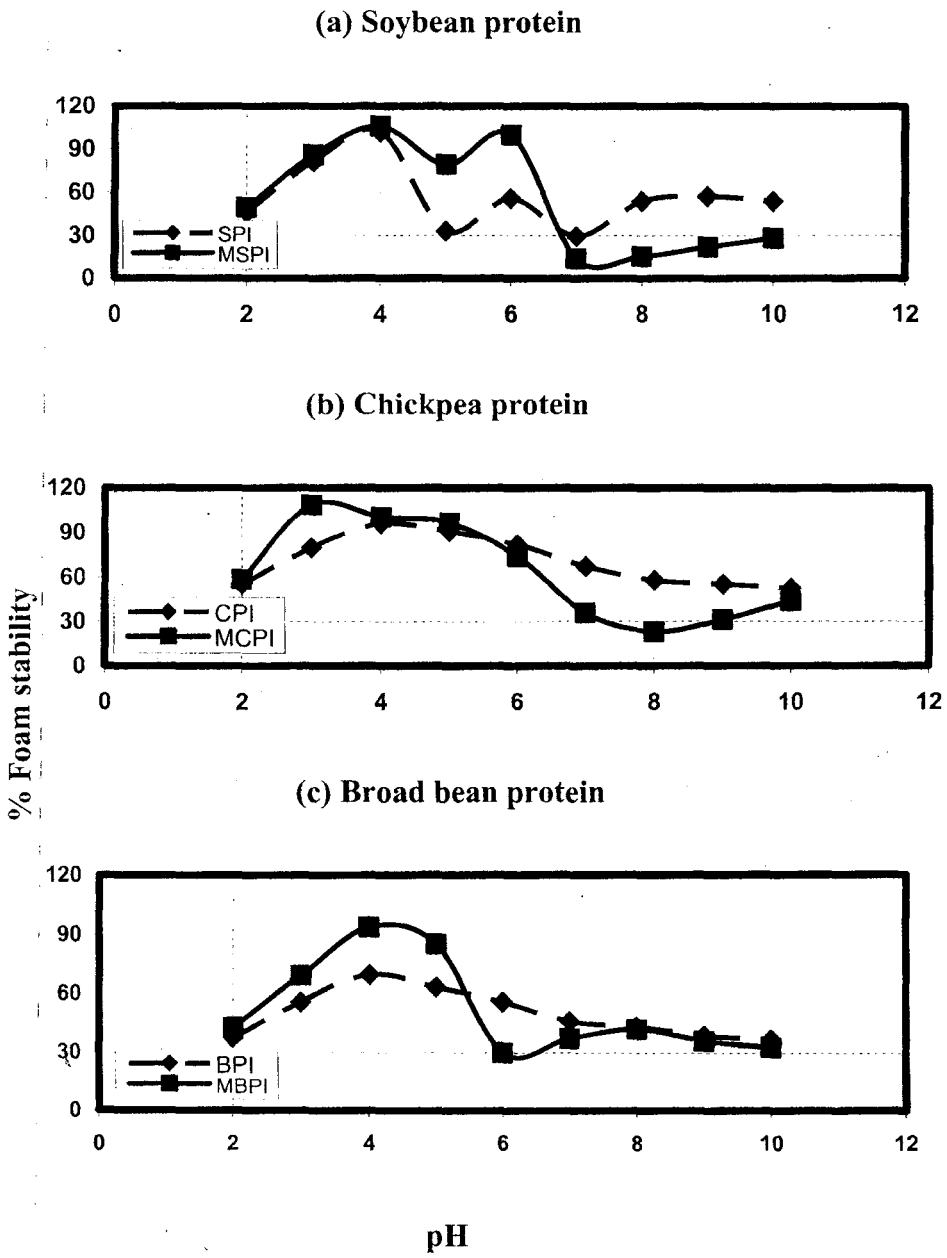


Fig.5. Foam stability of native and esterfied legume proteins at different pH values

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الخصائص الوظيفية لبروتينات البقوليات المعدلة كيميائيا بالاسترة

على عثمان محمد عثمان خليل- محمد مصطفى عفيفى عامر
محمـــود زكـــى ســـطوحى- على محمد على إبراهيم الجابى

قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة الزقازيق - مصر

بعد فصل المعزول البروتينى لبعض البقوليات (فول الصويا-الحمص-الفول البلدى) تم تعديلها كيميائيا على درجات مختلفة بالاسترة بالميثانول .تم قياس الخصائص الوظيفيـــة للبروتينات الطبيعية والمعدلة عند مدى من درجات الحموضة ٢-١٠ وهـــى متمثلـــة فـــى الذوبانية والخواص الاستحلابية والخواص الرغوية.وقد أوضحت البيانات زيادة فى ذوبانية البروتينات المعدلة عن البروتينات الطبيعية وذلك فى الوسط الحامضى عند درجة حموضـــة ٢-٦. وهذا التحسن فى الذوبانية يتناسب طرديا مع كلا من درجة التعديل وطبيعة البروتين المعدل. وكذلك أظهرت البيانات ان هناك زيادة واضحة فى الخـــواص الاســـتحلابية وكـــذلك الخواص الرغوية للبروتينات المعدلة عن الطبيعية فى الوسط الحامضى ايضا عنـــد درجـــة حموضة ٢-٦. وهذه الزيادة تتناسب مع درجة الذوبانية ودرجة التعديل وطبيعة البـــروتين المعدل.